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POLLEN DEVELOPMENT IN HYBRIDS OF *OENOTHERA LATA*×*O. LAMARCKIANA*, AND ITS RELATION TO MUTATION[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY LXXXIX

REGINALD RUGGLES GATES

(WITH PLATES II–IV)

This paper is a record, in part, of some investigations upon DEVRIES' mutants of Oenothera. The work was begun at the Marine Biological Laboratory, Woods Hole, Mass., in the summer of 1905; continued in the Botanical Laboratory of McGill University during the year 1905–6; and again at Woods Hole in the summer of 1906, where a larger garden of evening primroses was grown and studied, and material collected for further work; which is now being prosecuted at the Hull Botanical Laboratory, University of Chicago.

I have to thank Dr. B. M. DAVIS, under whose careful guidance the research was begun, for much attention and many suggestions and criticisms. I also have pleasure in acknowledging my indebtedness to Professor D. P. PENHALLOW, McGill University, for placing all the facilities of the Botanical Laboratory at my disposal, and for many kindly suggestions, to which Assistant Professor CARRIE M. DERICK also contributed, as the work progressed. The investigations at Woods Hole were carried on with the aid of scholarships from McGill University. I have to thank Dr. GEORGE T. MOORE for encouragement in the work of last summer, and also

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[1] A preliminary paper was presented before Sections F and G of the American Association for Advancement of Science at New York, December 28, 1906.

Mr. GEO. M. GRAY for aid in planting and caring for the garden. These researches are being continued with the aid of a fellowship at the University of Chicago, and I am indebted to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN for supervision.

Materials and methods

The first material of *Oenothera lata* was collected from a small garden of evening primroses at Woods Hole in 1905 from guarded seeds of DEVRIES. Stages were preserved for a study of sporogenesis and embryo sac development in *O. lata* and its hybrid forms; but the results recorded here will concern only pollen development, as the work on the megaspore and embryo sac has not been completed. The study of pollen development was taken up first because an interesting problem was presented by the abortion of the pollen, and because the reduction divisions in the microspore mother cell are for various reasons more easily investigated than those of the megaspore mother cell. The possibility of a cytological explanation of the segregation of characters in Mendelian hybrids, no one of which has yet been worked out on a chromosome basis, as well as the possibility of an explanation of the phenomena of mutation itself have also been kept in mind. The work has not yet reached a stage where it is possible to say that a cytological explanation for the Mendelian segregation of characters will be found, though this is possible. Some of the other results which will be described later, however, suggest that there may be found in the chromosomes a basis for the explanation of discontinuous variation or mutation, the suggestion being that the appearance of the mutant is preceded by certain morphological changes in the chromatin of the germ cells from which it originates, or, more exactly stated, that the germ cells from which a mutant arises differ in the structure of their idioplasm from the ordinary germ cells of the parent species.

Material has also been collected from *O. Lamarckiana* and several other of the mutants for comparison with *O. lata*.

The garden of 1906 at Woods Hole contained *O. Lamarckiana* and five of the mutants, all grown from guarded seeds of DEVRIES, which were planted in the greenhouse at the University of Chicago, the young seedlings being transplanted at Woods Hole about May 25.

By July 28, *O. rubrinervis* had begun blooming freely, and the other forms, including *O. lata*, began several days later. The earlier blooming of *O. rubrinervis* was very noticeable.

The history of the growth of these plants, which was observed with some care, will not be taken up here. Numerous crosses were made and other flowers self-pollinated for a study of the next generation, all necessary precautions being taken by means of bags, etc., to guard the flowers and to secure pure fertilization.

As stated by DeVries (**33**), the mutant *O. lata* does not mature its pollen and hence must be pollinated from another species, thus producing a hybrid in the second and succeeding generations. The seeds used were from a cross of *O. lata* with the pollen of *O. Lamarckiana*. These are said by DeVries (*l. c.* **1**:168) to segregate in the next generation, giving about 15–20 per cent. of *O. lata* plants and the remainder *O. Lamarckiana*, the characters of the latter thus being dominant.

My garden of 1906 contained fifteen plants from seeds of *O. lata* pollinated by *O. Lamarckiana*, ten of which conformed more or less completely to the characters of the pollen parent and four to the *lata* type, which is easily distinguished even in the rosette stage. One plant (no. 79), however, differed markedly from either of these forms, and was clearly a "mosaic" hybrid, *i. e.*, in some characters it resembled one parent and in some characters the other parent. The petals, however, were considerably larger than those of either parent species, and the sepals showed streaks of red, suggesting the sepals of *O. rubrinervis* but much paler. This character, however, is common to all the plants of *O. lata*×*O. Lamarckiana* having the *Lamarckiana* characters, and is occasionally seen to a less degree in *O. Lamarckiana* itself. The large ovaries and stout hypanthium, the greater pubescence on the young buds, and the broad leaves with their obtuse tips, are all characters of *O. lata*. But the leaves were scarcely at all crinkled (the more or less complete absence of crinkling being a character of *O. Lamarckiana* which distinguishes it easily from *O. lata*), and the general habit of branching and greater luxuriance of growth also correspond with *O. Lamarckiana*. This plant is mentioned as showing that segregation of the parental characters is not always complete in this cross, as this individual was fairly intermediate in position between the two parents, though no fractionization of any characters was observed.

In killing material various fixing fluids were used, the most satisfactory being 1 per cent. chromacetic, a chromacetic solution made up as follows: 1 per cent. chromic acid, 70^{cc}; glacial acetic acid, 0.5^{cc}; water, 30^{cc}; and the following chromosmacetic formula: 1 per cent. chromic acid, 70^{cc}; glacial acetic acid, 0.5^{cc}; 2 per cent. osmic acid, 5^{cc}; water, 30^{cc}.

Sections were cut varying in thickness from $3\,\mu$ to $10\,\mu$. The stains producing the best results were found to be Haidenhain's iron-alum-haematoxylin with orange G as a plasma stain, and Flemming's triple stain. The former is most satisfactory for the study of karyokinetic figures, particularly the chromosomes, but safranin and gentian violet give equally good results for synapsis stages.

Conditions of degeneration of pollen and tapetum in anther development

Unless otherwise stated, this account will refer to conditions observed in *O. lata*. The morphology of the *Lamarckiana* hybrid is very similar, so that the description would apply equally well to the latter, except where the pollen abortion in *O. lata* causes abnormalities.

Fig. 1 shows a transverse section of a single loculus in the "resting" pollen mother cell stage. The epidermal cells when normally developed have thick, highly cutinized walls at this early stage. Occasionally, when failure of anther development sets in at an earlier stage this cutinization does not take place, the cells remaining thin-walled. There are several "middle layers,"[2] generally about four. These are narrow radially and often show the effects of pressure from the large cells of the tapetum. The latter forms a well-organized layer of cells enclosing the sporogenous tissue. The granular appearance of the tapetal cells, owing to deeply-staining inclusions which fill the cytoplasm, easily distinguishes them from the middle layers. There are usually one to three longitudinal rows of pollen mother cells in a loculus, but the rows are not regular and a cross-section may show as many as five mother cells. In the great majority of cases the tapetum is normal at this time, but occasionally some of the tapetal cells or even the mother cells may be found degenerating in this "resting" condition; in *fig. 3* both these conditions are present. Certain scattered tapetal cells show by their loss of turgor and their deeply staining contents that they are degenerating. Their nuclei may become flattened, but at first retain their nucleoli and nuclear wall. Later the latter breaks down and the nucleoli lose their outline and become indistinct, until finally the nucleus has disappeared. These changes are frequently accompanied by vacuolation. The cytoplasm stains progressively deeper with safranin and with haematoxylin. This may be due to the distributed condition of the chromatin, which diffuses through the cell after the nuclear membrane disappears, or to the formation of some acid product in the cytoplasm as the result of the mucilaginous degeneration. Similar staining reactions are shown by degenerating mother cells. *Fig. 4* shows a stage of this degeneration.

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[2] There is some variation in the use of this term. It is here applied to all the layers between the epidermis and the tapetum, because they are undifferentiated and all have the same developmental history.

Most of the recent papers on pollen development in sterile hybrids do not describe the condition of the tapetal cells. GREGORY (**9**) mentions that the somatic cells surrounding the pollen mother cells in a hybrid race of *Lathyrus odoratus* are normal. JUEL (**13**), in his description of pollen development in the sterile hybrid *Syringa Rothomagensis*, makes no reference to the tapetal cells; nor does CANNON describe the condition of the tapetum in his two papers (**4, 5**). TISCHLER (**31**) finds conditions in the tapetal cells of hybrids of Ribes very similar to those described in some detail by BEER (**3**) in *Oenothera biennis* and *O. longiflora*, and which I have observed also in *O. lata* and *O. Lamarckiana*.

The tapetal cells in *O. lata* are uninucleate during the "resting" stage of the pollen mother cells. During the early presynaptic stages in the mother cells the tapetal nuclei divide mitotically, and the binucleate condition persists for some time. This first nuclear division in the tapetal cells may take place almost simultaneously throughout a loculus, so that many mitotic figures in various stages are often found in the same section. Later divisions are more irregular and are probably chiefly by amitosis, as stated by BEER (**3**) for *O. longiflora* and *O. biennis*, although occasionally two spindles may be found in the same tapetal cell.

My observations on the later stages of tapetal development agree in the main with those of BEER, and so will not be taken up here in detail. In some cases appearances suggest simultaneous division of a nucleus into several smaller nuclei, the latter often forming closely aggregated masses, as figured by BEER. At a still later stage, when the persistent pollen grains have begun to assume their adult shape, fusion of these masses of nuclei into one or two apparently occurs, and the tapetal cells return to a binucleate or even uninucleate condition. Both nuclei and cytoplasm now, however, show a very different appearance from the early uninucleate and binucleate condition, the nuclei being at this time much larger and having extremely little chromatic content. The cytoplasm, which is loosely reticular rather than granular at this time, frequently contains more or less indefinite and irregular chromatic masses. BEER describes the origin of these masses and compares them with the chromidial structures found by MEVES (**19**) in the tapetal cells of *Nymphaea alba*, and with similar bodies found by GOLDSCHMIDT (**8**) in the cytoplasm of certain animal tissues. These tapetal cells of the genus Oenothera offer excellent material for a further study of amitosis. It is interesting to note that the structure of the tapetal cells in the mutant *O. lata* is apparently identical with that of the well-known members of the genus, such as *O. biennis*. During these later nuclear divisions of the tapetal cells in *O. lata*, radial elongation of the cells may take place, but rarely enough to nearly fill the cavity of the loculus left by the degeneration (in such cases) of the mother cells. Transverse divisions of the tapetal cells are comparatively rare.

In the great majority of cases the tapetal cells begin to break down before disintegration in the mother cells sets in. As already stated, this disintegration may appear as early as the resting stage of the mother cells, where scattered cells begin to lose their turgor and degenerate. In later stages of development a great variety of conditions of disintegration of this tissue may be found. The tapetum may break down irregularly in different parts of a given loculus, or only on one side, or in patches. Different loculi of the same anther and different anthers of the same flower show wide variations in this matter.

POHL (**22**) states, followed by DEVRIES (*l.c.* **1**:292), that in *O.*

lata pollen development is normal up to the tetrad stage; that the tapetal cells later elongate and multiply, filling the whole loculus; and that the pollen cells in the mean time have broken down and disappeared, only a few small scattered grains remaining. The first statement is erroneous, because degeneration may take place in the pollen mother cells before the reduction divisions (*figs. 3, 4*). POHL'S account of the history of the tapetal cells is also contrary to my observations. It is true in general that the tapetal cells continue active during synapsis and the reduction divisions in the pollen mother cells, although breaking down frequently begins at this time, or even earlier, in the "resting" stage, as already mentioned. But in later stages, when the pollen cells have largely disappeared after the reduction divisions, leaving an empty cavity, the normal condition is not as POHL states—ingrowth of the tapetal cells to fill the cavity—but partial or complete disappearance of the tapetum; and in the latter case *subsequent ingrowth* of the middle layer cells behind to fill more or less completely the cavity (*figs. 5, 6*). Stages of this process are found in which the disintegrating tapetal cells form a thin layer lining the locular cavity (which is usually empty under these conditions), the remains of this layer sometimes appearing later as a thin, homogeneous, deeply staining ring (*fig. 5*), which may afterwards completely disappear. The middle layer cells, which now line the cavity, in the meantime become rounded on their inner surfaces, owing to the release of the pressure of the tapetal cells. They then push in to fill the cavity and may even multiply by mitotic division. The cavity of the loculus may thus become completely closed (*fig. 6*).

POHL'S error in mistaking these middle layer cells for tapetum was probably due to a hasty and insufficient examination, for in carefully prepared sections the former have a totally different appearance from the tapetal cells, for which they could not possibly be mistaken. While the latter are easily recognizable by their densely granular contents and conspicuous nuclei, as well as by their rectangular shape, the middle layer cells have no granular contents, are uninucleate, with small nuclei having little chromatin, and are rounded and irregular in shape. POHL'S error is probably to be attributed to his failure to observe the degeneration and disappearance of the tapetum before the ingrowth of the middle layer cells takes place, as well as to distinguish between the cells of the middle layers and the tapetum by their appearance.

This condition, where the tapetum breaks down early and the middle layer cells then grow in and fill up the loculus, is common and may occur in one loculus of an anther when the adjoining loculi show normal functioning tapetum. The ingrowth is frequently irregular, occurring more rapidly on one side of the cavity, thus causing the latter to lie excentrically in the anther lobe and to assume a narrow and flattened or irregular shape. This ingrowth of middle layer cells does not initiate the disintegration by pressure on the tapetal cells, or in any other way, for it only begins after the tapetum has more or less completely disappeared, which is long after the first appearance of degeneration in the tapetal cells. That the tapetal cells are necessary for the nourishment and growth of the pollen grains is shown by the entire absence of the latter in such cases long before the cavity is obliterated.

Another and perhaps more common condition is for the tapetum to persist through the tetrad divisions and until the persistent pollen grains attain a large size. The loculus meantime enlarges considerably by growth (*fig. 7*), and also by the flattening and breaking down of the middle layers, as would be expected in normal pollen development. The tapetal cells also generally become flattened at this time, and stretched out so as to line the larger cavity created partly by growth and partly by the degeneration of the middle layers. In such loculi pollen grains in various stages of development are frequently though not always found, although when present they almost invariably assume irregular and bizarre shapes, though they may contain nuclei which are normal in appearance. Rarely pollen grains may be seen which have the triangular disk-shaped appearance characteristic of the pollen in this genus; but such grains have never been observed to reach full size, although abnormally shaped grains may do so sometimes.

It is evident that the break-down of the middle layers, as mentioned in the last paragraph, is due to pressure from the functioning tapetum, for the cells of the middle layer become flattened radially and lose their contents, while the tangential walls may persist for some time, forming irregular concentric rings of dark-staining substance. Later these completely disappear, leaving no trace of the middle layers. The tapetum then lies directly against the epidermis.

The presence of pollen grains in various stages of development when the tapetum persists, and their complete absence when the tapetum disappears early, shows that the failure of the tapetum to develop is at least one factor in the abortion of the pollen. POHL's conclusion that "die Zellvermehrung und relative Persistenz der Tapetumzellen hemmt die Pollenentwicklung" is evidently an error, for, in the great majority of cases at least, where the tapetum persists, it does not show abnormal radial elongation or growth to fill the locular cavity, the tapetal cells in older stages becoming instead very much flattened radially, as already seen. The cases where the tapetum does not persist, but where the cells of the middle layers grow in and obliterate the cavity, have already been described. In short, when the tapetum persists the middle layers break down, as is the usual condition in normal pollen development; but when the tapetum degenerates at an abnormally early stage, the cells of the middle layers grow in and fill the loculus.

History of the pollen mother cells

PROPHASE AND SYNAPSIS

The description thus far has dealt chiefly with the general morphology of pollen development in *O. lata;* and the development of the pollen mother cells will now be taken up in greater detail. It is a noteworthy fact that the mother cells of a given loculus show but little variation in their stage of development. Generally, however, there is a slight progression from top to bottom of a loculus; *e. g.*, when the pollen mother cells at the top of a loculus are in pre-synaptic stages, those at the bottom may be in the late post-synaptic stages of the prophase; or when the mother cells at the top of a loculus are in metaphase of the heterotypic division, those at the bottom may have reached the telophase with reconstructed nuclei. In comparing different loculi of an anther or different anthers of the same flower, the variation is often much greater. Thus in some loculi the pollen mother cells may be in synapsis; while in others of the same or another anther in the same flower, they may be in metaphase of the heterotypic division. This irregularity is probably connected with the failure in pollen development.

The cytoplasm of the mother cells in the "resting" condition contains numerous large spherical starch grains. In safranin-gentian they stain pale blue and the central hilum is made more prominent by its retaining a highly refractive globule of xylol. These starch grains are thus very conspicuous objects in the mother cells (*fig. 3*). With iodine they appear brown and angular, and the iron-haematoxylin stain leaves them invisible, so that very different appearances of the mother cells are obtained according to the method of treatment. During the prophases of the first mitosis the starch grains become more abundant, until the cytoplasm is literally packed with them. This reserve store of nutriment is used up during the reduction divisions, and no starch grains are to be seen in the pollen tetrads. In iron-haematoxylin the cytoplasm of the pollen mother cells frequently presents a rather obscurely radiate appearance during the pre-synaptic stages. In the earlier or "resting" condition of the nuclei in the mother cells they contain one large nucleolus, which is commonly placed near the nuclear wall and contains one to several vacuoles of varying size with highly refractive walls (*fig. 9*). In addition, two or more smaller chromatic bodies are usually found distributed near the nuclear membrane. They appear connected and often surrounded by the delicate reticulum scattered through the nucleus. The large nucleolus is several times the size of the smaller bodies. Sometimes it is in the center of the nucleus, and the smaller bodies are appressed to the nuclear wall. Vacuoles are usually observed only in the large nucleoli, but occasionally minute ones may be seen in the smaller chromatic bodies. More rarely two large nucleoli of nearly equal size may be found. The small round chromatic masses arise from the more angular chromatic bodies found at the nodes of the reticulum in an earlier stage (*fig. 8*). These bodies probably correspond to OVERTON'S "prochromosomes," though they have not been studied fully. The next stage leading towards the synapsis condition is apparently the beginning of contraction of the nuclear reticulum. The nuclear membrane is often extremely delicate at this time, and as indicated in *fig. 10*, the nucleolus is frequently surrounded by a compact reticulum which has contracted from the nuclear membrane, and in which are embedded, especially near the margin, the "prochromosomes," forming an incomplete circle around the periphery. The material in which are found the beginnings of this contraction, as well as its later stages, was fixed and stained in the same manner as the slightly earlier stages in which it does not occur.

The transition from this stage to the regular spirem stages has not been followed. The pre-synaptic stages with a continuous spirem last for a comparatively long time, as shown by the frequency of their occurrence. The spirem is at first extremely delicate, very long and tangled (*fig. 11*). One large nucleolus is always seen at this time, but one or more smaller ones may also be present. In a later stage the nucleolus is for a time closely appressed and somewhat flattened against the nuclear wall. Loops of the spirem extending to the wall frequently give a false appearance of doubling (*fig. 14*), but though a great many spirem stages have been studied, a double thread has never been observed. The small size of the nuclei, however, and the delicacy of the spirem thread make it very difficult to determine this point.

The spirem gradually contracts into a dense ball with a few loose threads projecting irregularly (*fig. 12*). In this closely contracted condition it may form a body about the size of the nucleolus, which can only be distinguished from the latter by its somewhat irregular outline (*fig. 13*). In the next stage observed the spirem is again loosely arranged in the nuclear cavity, but is greatly contracted in length and several times the thickness of the original spirem before the contraction stage. This is shortly before the spirem breaks up into chromosomes, and the thread is at this time about the same thickness as the chromosomes in the later prophase. Throughout the earlier stages of the prophase the spirem thread is continuous, and, judging from its frequency, the duration of the long delicate spirem previous to the synapsis stage is considerably longer than that of the short thick spirem after synapsis and before it segments into chromosomes. In the latter stage, before segmentation into chromosomes, there is frequently found, besides the spirem, a ring-shaped body of chromatic material exactly like the spirem in thickness and staining power. This has evidently been cut off from the spirem (*figs. 17, 18, 19*). Frequently there are two such rings.

Fig. 16 probably shows the origin of one of them by the cutting off of a loop of the spirem. There can be no doubt that these bodies arise from the spirem thread previous to the breaking up of the remainder of the thread into chromosomes. The nucleoli at this time are large, globular, and deeply-staining. *Fig. 20* shows a slightly later stage, in the prophase, where two of these ring bodies, which I shall call "heterochromosomes"[3] on account of their later history, are present in addition to the nucleolus and several chromosomes. That these are not the ring-shaped chromosomes frequently found in the prophase of the heterotypic mitosis is shown by their later history; for the rings do not segment in metaphase, forming V- or U-shaped structures, but pass towards the poles of the spindle or into the cytoplasm undivided and retaining their ring form. The shape of the ordinary chromosomes, which are nearly globular or slightly elongated and pear-shaped, precludes the possibility of the pairs forming the X, Y, V, and O shapes which characterize the heterotypic mitosis when the chromosomes are long and rod-shaped.

The heterochromosomes are not always formed, however, and hence if the original pollen mother cells were all alike in the early synapsis stages, two kinds of pollen cells must result; for, as will be shown later, the heterochromosomes probably represent a portion of the chromatin which is rejected and disappears in the cytoplasm, so that the daughter nuclei must differ in their chromatin content. *Fig. 21* shows thirteen chromosomes in the prophase at a later stage, when they are condensed to a considerably smaller size. The adjacent section shows a nucleolus and one chromosome, but no heterochromosomes are present. It has been determined from a number of counts in the prophase that the sporophyte number of chromosomes, exclusive of the heterochromosomes (or at least when the latter are not present), is fourteen. Whether the number is the same when the heterochromosomes are present has not yet been determined with certainty, but it appears probable that it is less. BEER (3) states that the reduced number of chromosomes in *O. longiflora* is seven; hence these two species probably agree in the number of their chromosomes. Frequently the chromosomes in the late prophase show evident pairing, but it is usually difficult to represent this accurately in a drawing, because the members of a pair generally lie in different planes. However, indications of such a pairing are shown in *figs. 21* and *22*. The positions of the chromosomes make it certain that this is an apposition and pairing of whole chromosomes and not a splitting. This is evident, for the chromosomes are completely separated when first seen in prophase and are only arranged in pairs later, on the equatorial plate.

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[3] The term was proposed by MONTGOMERY as a general term for the aberrant chromosomes in Hemiptera, and is used in preference to "idiochromosomes," because the latter term has been applied to a certain type of heterochromosomes by WILSON (37). More recently MONTGOMERY (20) has proposed the term "allosome" as a general term for all aberrant chromosomes, and "monosome" for one which passes undivided to one of the daughter nuclei. The latter term would be most suitably applicable to the bodies described here.

Fig. 22 shows another prophase with ten chromosomes, no heterochromosomes, and a nucleolus in which the stain has been withdrawn from its outer shell except on one side. This condition of the nucleolus is common enough to cause one to suspect that it possesses some significance. Possibly the side of the nucleolus in which the amphipyrenin retains the stain is the one which was earlier attached to the nuclear wall. However I have no observations to prove this. The nucleolus now rapidly loses its staining power, and in the late prophase, after the chromosomes are formed, frequently stains only with orange G in an iron-haematoxylin-orange stain. It gives no indications of loss of shape or size before it disappears, and I think the most probable supposition is that it is quickly dissolved in the cytoplasm when the nuclear membrane breaks down. The evidence is clear that it disappears about this time, and it is very rarely seen as late as metaphase. I may state here that the stages of its reappearance in the reconstructed daughter nuclei at the end of the heterotypic mitosis are in the reverse order of its disappearance. Soon after the nuclear walls appear around the daughter nuclei, when the nuclear sap has increased in quantity, the nucleoli appear (*fig. 42*) as pale yellow-staining bodies, usually several in number and frequently attached to the nuclear wall. It is impossible to believe that these bodies are reconstituted from fragments of the original nucleoli thrown out into the cytoplasm during the first mitosis and collected again into the daughter nuclei, as described by SCHAFFNER (26) for the megaspore divisions in *Lilium philadelphicum*. For in the last stage of their disappearance they retain their shape but lose their staining power, as if the deeply staining substance (chromatin ?) had been dissolved away, leaving the yellow-staining ground-substance, and in the first stage of their reappearance the yellow-staining material only is present. I think this precludes the possibility of dentifying the bodies in the cytoplasm, which stain like chromatin, as fragments of the nucleolus.

THE HETEROTYPIC MITOSIS

Thus far the description of events in the pollen mother cell has related only to *O. lata*, because the corresponding stages in the *O. Lamarckiana* side of the hybrid have not been studied. There is every reason to believe, however, as will be shown later, that the history is the same except for the difference in the number of chromosomes, because heterochromosomes are found very commonly in the *O. Lamarckiana* side of the cross, in the metaphase of the heterotypic mitosis, and they doubtless have the same origin as in *O. lata*. These heterochromosomes are the first described in plants, and a discussion of their possible significance will be deferred to the end of the paper.

Fig. 23 shows a metaphase of the heterotypic mitosis in *O. lata*. There are shown twelve chromosomes (some of which are in pairs of equal size) and a heterochromosome. *Fig. 24* shows two heterochromosomes of unequal size far out in the cytoplasm.

Figs. 25 and *26* are from sections of spindles showing the heterotypic mitosis in the *O. Lamarckiana* hybrid. In *fig. 25* the heterochromosomes are of equal size and are found on the same end of the spindle. This condition is very commonly found. In *fig. 26* the heterochromosomes are in the cytoplasm and not attached to the spindle; one of them is larger than the other and they are apparently proceeding towards opposite poles of the spindle.

Fig. 27 is a side view of a spindle showing a small chromosome out of position near the equatorial plate. A similar body is seen in *fig. 26*. What significance these may have is not known. Their early history has not been observed. They are not ring-shaped, like the typical heterochromosomes, but they frequently stain less deeply than the regular chromosomes. These bodies were only observed a few times, while the heterochromosomes are very common although they are certainly absent from some pollen mother cells.

Figs. 29, 30, 31, and *32* are sections of the equatorial plate of the *O. Lamarckiana* hybrid, showing one or more heterochromosomes. The chromosomes are so densely grouped at this time that it is often impossible to make out the individuals. This renders some of the figures unsightly, but they are intended to be merely accurate representations of what is seen on the spindle.

After passing into the cytoplasm, where they are generally found in metaphase, the heterochromosomes apparently degenerate and disappear. They gradually become smaller and stain less deeply, though retaining their ring form, as in *fig. 32.* Finally they are so small and inconspicuous as to be scarcely distinguishable from the granules (microsomes) of the cytoplasm. It is probable that they usually disappear before the telophase of the heterotypic mitosis. Several other points in regard to their history have yet to be determined.

Figs. 34 and *35* are equatorial views. *Fig. 34* shows several chromosomes together with a heterochromosome, and also a very large or giant ring chromosome, which apparently contains enough chromatin for several chromosomes. *Fig. 35* shows another; and these two cases were the only ones observed. They are apparently quite distinct from the heterochromosomes already described, although they are ring-shaped. No other observations have been made in regard to them.

Fig. 35 is a polar view of the equatorial plate of the heterotypic mitosis in the *O. Lamarckiana* hybrid. The apparently unequal pairs are probably due to foreshortening, owing to one chromosome lying in a lower plane. *Figs. 36* and *37* are early anaphases in the *O. Lamarckiana* hybrid. *Figs. 38* and *39* are late anaphases. *Fig. 38* shows clearly the premature splitting of the chromosomes for the homotypic division. This splitting may occasionally be observed in late metaphase, and in the late anaphase the separation of the halves is often complete. V-shaped chromosomes were not observed, the split apparently taking place simultaneously throughout. Thus it is clear that the heterotypic mitosis distributes whole chromosomes, which were arranged in pairs (though not at first in contact) in the prophase; while the homotypic mitosis is a typical division, separating the halves of the chromosomes already formed by splitting in the anaphases of the heterotypic division. The heterotypic is thus the reducing division. The shape of the chromosomes of course makes it impossible to determine whether the second splitting is longitudinal or transverse.

Fig. 40 shows a telophase in which the reconstruction of the daughter nuclei has begun. Delicate nuclear walls are appearing and the chromosomes have assumed angular, lobed, and irregular forms, but very little nuclear sap has yet appeared. Materials staining like chromatin are scattered through the cytoplasm. *Fig. 41* shows the same stage, in which bodies as large as chromosomes and taking the same stain are scattered through the cytoplasm (particularly in the region of the daughter nucleus). It has already been shown, I think, that these bodies cannot be fragments of the nucleolus. Neither are they likely to be fat bodies, since the cytoplasm in earlier stages contained abundant storage nutritive materials in the form of starch. It seems then most reasonable to conclude that these bodies are actually chromosomes, and fragments of chromosomes which for some reason have become separated from their fellows on their way to the poles of the spindle. It is difficult to understand, however, what forces carry them into the cytoplasm, for among hundreds of spindles studied no irregularities in their structure have been observed which would cause the chromosomes to be distributed irregularly. Indeed the heterotypic spindle is notably regular in form. The earlier stages of spindle formation have not been studied, however, and it may be that these would account for some of the chromosomes not being on the spindle. It is doubtful if the heterochromosomes are ever attached to the spindle. Of course the position of chromosomes near the nuclear membrane is easily accounted for by their having merely failed to pass to the poles of the spindle, but this will not explain the position of many which are found scattered in the cytoplasm throughout the cell and frequently near its periphery.

Fig. 42 is a polar view of a daughter nucleus in a later stage of reconstruction. The nucleus has grown greatly in size, owing to a very rapid increase in the nuclear sap. At the same time the chromosomes have become drawn out into irregular shapes, are much reduced in size and stain less deeply. Several nucleoli have appeared, but they do not take the chromatin stain. In an iron-haematoxylin-orange stain they take only the orange; thus it is difficult to see how the view that the nucleoli of the daughter nuclei are reconstructed from fragments of the former nucleolus (SCHAFFNER **26**) can apply here, for fragmentation of the nucleoli before their disappearance has never been observed, and their disappearance is preceded by loss of staining power, as if the chromatic-staining substance were first dissolved away, leaving the pale ground-substance. The method of final disappearance of the latter has not been observed. As already mentioned, the reverse series of events occurs in the reappearance of the nucleoli in the daughter nuclei. When first observed in the daughter nuclei, they have their characteristic size and shape, but stain yellow and only acquire the chromatic stain later. They were never observed until there was a considerable accumulation of nuclear sap, and are certainly not visible until some time after the membranes of the daughter nuclei have been formed. These changes in the staining power of the nucleoli might be attributed to chemical changes in the nuclear sap, the medium in which they float, such as would alter the staining reaction of bodies contained in it. But while the nucleolus undergoes these changes before its disappearance, the chromosomes remain constant in their staining properties, which makes it appear more probable that the change in staining is due to actual loss of material from the nucleolus.

Several of the figures (*figs. 37, 38, 39*) show that the number of chromosomes in the *O. Lamarckiana* hybrid is greater than in *O. lata*. A count has not been made in the prophase, but from numerous counts in the metaphases and anaphases, the sporophyte number is found to be at least twenty and probably very near that number (*fig. 37*). It will be remembered that the sporophyte number in *O. lata* is fourteen. The bearing of these facts will be discussed later.

The study of several other interesting features of the telophase of the heterotypic as well as of the homotypic division has not yet been completed.

The pollen tetrads

The pollen tetrads in *O. lata* show numerous interesting features. As already stated, development to this stage frequently takes place, though not always. When development takes place, the tapetum is also found to be normally developed and functional. In some cases, as in *figs. 43* and *44*, the normal number of four nuclei is found (three shown in the figures), though these may vary in size (*fig. 43*) and in chromatin content. In other cases (*fig. 45*) chromatic bodies are found in the cytoplasm. In *fig. 46* one of the nuclei has two others lying near it. *Fig. 47* shows a tetrad with extra nuclei. Occasionally large and irregular shaped nuclei are found, as in *fig. 51*. *Fig. 48* is a section of a tetrad showing an extra nucleus formed from a single chromosome and connected by spindle fibers with one of the larger nuclei. *Fig. 49* is a portion of a tetrad showing a nucleus with a smaller one beside it; and *fig. 50* similarly shows two small extra nuclei, each containing two chromosomes. Pairing of the chromosomes in the large nuclei of the tetrad is sometimes seen. Chromosomes are frequently found just outside the nuclear wall; this is probably due to tardiness in passing to the poles of the spindle. Such chromosomes may or may not be surrounded by a "nuclear membrane." Most of the pollen tetrads show evidences of breaking down, and a great many are more or less completely degenerated. In the *O. Lamarckiana* hybrid irregular-shaped pollen grains are quite common in some anthers; *fig. 52* shows some of these irregularities. There may be extra lobes or fewer lobes than the normal, and they may be irregularly placed. Occasionally complete sterility of certain anthers of a flower may be found in the *O. Lamarckiana* hybrid, as in *O. lata*.

Discussion

A full discussion of the literature bearing on the facts set forth in this paper will not be attempted at this time, as the work touches upon several distinct fields. But I shall mention a few of the most important papers bearing directly upon these investigations, and close with a short discussion of the general bearing of the most important results.

THE EXTRA NUCLEI IN POLLEN TETRADS

Wille published a paper (**34**) on pollen development in the angiosperms in 1886, before botany had acquired its present technique, in which he cites a list of cases of extra nuclei in the tetrad divisions. He remarks that these are chiefly cultivated plants, and suggests that the extra nuclei are indications of greater variability in structure resulting from cultivation. This was of course before the modern views of chromosome reduction and alteration of generations had been developed. The fact that extra nuclei are so commonly found among the irregularities in the divisions of the pollen mother cells in hybrids, together with the well-known fact that cultivated plants are very commonly hybridized, would seem to lay open to suspicion the purity of any species in which such irregularities occur. Even *Hemerocallis fulva*, which is commonly quoted as a pure species having these irregularities, is well known to have been long under cultivation, and three varities of it are described by BAILEY (**2**). The other members of the list given by WILLE (*l. c.* 60), with mention of their cultivation and varieties, taken from BAILEY'S *Cyclopedia of American Horticulture*, are given below.

Name	Varieties	
Ampelopsis hederacea =A. quinquefolia	. .	cult. climbing vine
Azalea indica	. .	cult. and hybridized
Begonia sp	. .	cult. and variable
Charitzania ilicifolia	. .	not in Bailey
Cornus sanguineus	2	cult. shrubs
Elatine hexandra	. .	not in Bailey
Ficaria ranunculoides	. .	not in Bailey
Fuchsia sp	. .	cult., very variable, and probably hybridized
Funkia ovata	1[4]	cult.
Hemerocallis fulva	3	cult.
Lonicera coerulea	. .	cult. shrubs
Prunus cerasus	. .	cult. and hybridized
Rumex patientia	. .	cult. and nat.
Scleranthus annuus	. .	not in Bailey
Stellaria glauca	. .	not in Bailey
Symphytum officinale	1	cult.
Syringa persica	2	cult.

An examination of the list shows that all but five are forms cultivated in America, and most of these are either open to suspicion as hybrids or known to have been hybridized. In the light of these facts, a careful examination of the history of all forms in which such extra nuclei are known to occur would be advantageous. It would thus be possible after a time to decide whether they result from hybridization only, or whether they may be the result of some other constitutional derangement caused by cultivation. It should be mentioned in this connection that Miss LYON (16) described five and six spores in a mother cell of *Euphorbia corollata*, which is a common species in this region. In this case the daughter cells are stated to be all of the same size, however, which suggests that the number may have been produced by extra divisions of one or more of the daughter cells, rather than by some of the chromosomes becoming scattered in the cytoplasm. The same explanation may apply to some of the other forms mentioned; and it is also necessary to assume extra divisions in cases such as *Lonicera coerulea*, where as many as fourteen daughter cells are figured in one mother cell. FULLMER (7) thought that such extra mitoses would account for most of the conditions in Hemerocallis, but in this he was probably incorrect.

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[4] Two other varieties are given by J. G. BAKER in "A revision of the species of herbaceous gamophyllous Liliaceae" in Jour. Linn. Soc. 11:366. 1871.

A more careful study of some of these forms, and of some of those said to produce only one, two, or three pollen grains from a mother cell, as described in the paper of JUEL (14) on Carex, would doubtless furnish many interesting results and aid in a determination of the causes of these irregularities.

THE PHENOMENA OF POLLEN DEGENERATION

During the last few years a number of cases of sterility in the pollen of hybrid plants have been described, with interesting morphological and cytological results. JUEL (13) in 1900 described pollen development in the sterile *Syringa Rothomagensis*, which is a cross between *S. vulgaris* and *S. persica*. He also studied the corresponding stages in the parents for comparison. In *S. vulgaris* only about 50 per cent. of the pollen grains were found to be normal, and in *S. persica* good grains were few. Practically the same conditions of development were found in *S. persica* as in the hybrid. A great variety of irregularities in the reduction divisions was found in the hybrid. As in *O. lata*, the degeneration sometimes began as early as the synapsis stage, but generally the tetrad divisions were completed. A drawing apart of the nucleus into two may occur in the spirem stage or after the spirem has segmented into chromosomes. The latter at least could not be called amitosis. In the first reduction mitosis chromatin appears in the cytoplasm, as in the Oenothera hybrids studied. The spindle is also sometimes multipolar and irregular. Several nuclei may be found in a tetrad, and also chromatin scattered in the cytoplasm, as in *O. lata*. This hybrid shows a greater variety of irregularities than have been observed in any other hybrid described.

GUYER (**10, 11**) found in the spermatogenesis of hybrid pigeons similar conditions with extra and irregular spindles and incomplete pairing of the chromosomes. CANNON (**5**) found in hybrid cotton, *Gossypium barbadense* × *G. herbaceum*, that degeneration of the pollen mother cells sometimes begins in the resting stage previous to synapsis. The divisions in some of the pollen mother cells are amitotic, the nucleolus dividing first, followed by equal or unequal segmentation of the nucleus and its reticulum. In the case of equal segmentation, pollen tetrads of normal appearance may be formed. Multipolar spindles, with many small nuclei, were also found. In fertile hybrids of certain races of peas, which obey Mendel's law, CANNON (**4**) found no difference in pollen development between the hybrids and their parents. He states that there is a pairing of the chromosomes in the telophase of the last sporogenous division previous to synapsis. It should be said, however, that these two papers are not as complete and satisfactory as some of the others.

ROSENBERG'S (**23, 24**) study of pollen development in *Drosera longifolia obovata*, a hybrid of the intermediate type, between *D. rotundifolia* and *D. longifolia*, but nearer *D. longifolia*, shows that some of the smaller chromosomes of *D. longifolia* are left behind in the cytoplasm in the first and second divisions, where they also form small nuclei. Later the greater number of the pollen grains lose their contents. The embryo sac development also usually stops at the binucleate stage. The sterile hybrid sweet peas (*Lathyrus odoratus*) studied by GREGORY (**9**) show an earlier degeneration of pollen than the other cases cited. Portions of the spirem are often destitute of chromatin and the chromosomes frequently are pale, which GREGORY attributes to lack of nutrition. The nucleolus, he thinks, serves to store most of the chromatin between the reduction mitoses. Other conditions simulating amitosis in the pollen mother cells are illustrated. Degeneration of the pollen mother cells usually takes place in the prophase, but occasionally the first spindle is formed, though it is irregular. Development never proceeds to a later stage. The sterility is here a Mendelian character, recessive and correlated with a certain somatic character, the two always appearing together.

TISCHLER (**31**) ascribes the sterility of the hybrids of Ribes and Bryonia, as well as those of *Lathyrus odoratus*, to the influence of long culture. He studied three hybrids of Ribes: (1) *R. intermedium* = *R. sanguineum* × *R. nigrum;* this hybrid is fertile, producing 10–15 per cent. good pollen grains; (2) *R. Gordonianum* = *R. aureum* × *R. sanguineum;* absolutely sterile and showing great vegetative luxuriance; (3) *R. Schneideri* = *R. grossularia* × *R. nigrum;* sterile. He finds a splitting of the spirem in the strepsinema stage, and that the chromosomes when formed lie in pairs in the prophase. As in *O. lata*, the heterotypic is a reduction division, the homotypic an ordinary equation division, and the chromosomes split (longitudinally) in the anaphase of the first mitosis. In all the hybrids, as well as in the parents, *e. g., R. sanguineum*, the reduction divisions are mostly normal, though in *R. Gordonianum* occasional extra spindles are found in the cytoplasm; otherwise its tetrad divisions cannot be distinguished from those of *R. intermedium;* but the sterile grains do not grow in size. The first evidences of degeneration appear then in Ribes later than in any of the others mentioned, *i. e.*, usually after the reduction divisions are completed.

In a sterile hybrid of *Begonia alba* ♀ × *B. dioica* ♂, TISCHLER (**32**) finds that when the reduction divisions in the pollen mother cells are irregular, they are invariably preceded by abnormalities in synapsis; but that when synapsis is accomplished regularly the reduction divisions are also carried through without irregularities. There is often unequal division of the chromosomes on the heterotypic spindle. In the tetrad divisions, chromosomes in the cytoplasm and extra nuclei are relatively seldom found. The pollen grains are very often degenerate and of unequal size. In the female gametophyte the megaspore mother cells divide normally, but the embryo-sac mother cell degenerates. Whether another embryo-sac is formed later from the sporophyte tissue, as shown by TISCHLER for *Cytisus Adami* (**29**), *Ribes Gordonianum*, and *Syringa chinensis* (**30**), has not been determined.

In some of the papers sufficient attention has not been given to a comparison of the adult characters of the hybrid with those of the parents, *i. e.*, whether the hybrid is a mosaic, Mendelian, an intermediate, or goneoclinic to one parent, constant and invariable or breaking up into different types, etc. It would be particularly valuable to know in every case to what particular type the hybrid whose germ cells are studied conforms, and what variations if any occur.

THE POSSIBLE CAUSES OF STERILITY IN HYBRIDS

That hybrids are frequently sterile has of course long been known and commented upon. The recent morphological and cytological papers already cited have not reached an explanation of its cause, though various suggestions have been made. Some of the causes suggested may be classified into several general categories which are not, however, necessarily mutually exclusive: (1) lack of "nutrition" in the parts affected, causing, *e. g.*, failure in development of the tapetum or low staining capacity of the chromatin in the germ cells; (2) the influence of long culture (whatever that may be); (3) irregularities of development in the germ cells, appearing during synapsis or the reduction divisions and due to some lack of harmony (variously expressed) between the idioplasms; (4) some more deep-seated phenomenon affecting the physiology of the whole plant. This is not an attempt to classify all the possible causes of sterility suggested, but it will be seen that the factors are stated from widely differing points of view and with varying amounts of definiteness. Some of them might be viewed as effects rather than as causes.

In *O. lata*, if the tapetal cells always showed a degeneration before the pollen mother cells, we might conclude that the failure of the tapetum to function (in the secretion of nutriment for the pollen grains, which is generally conceded to be its chief function) was the immediate cause of the abortion of the pollen. But this is not always the case; for degeneration in the pollen mother cells may appear as early as in the tapetal cells or earlier; while GREGORY states that in the hybrids of *Lathyrus odoratus* the "somatic cells" are normal throughout. GUYER (**10**) suggested in 1900 that incompatibility of the chromosomes or plasms, as shown in the prophases of the first reduction division, is the source of the subsequent irregularities in spermatogenesis, and consequently the cause of sterility in hybrid pigeons. But in the case of *O. lata* such an explanation can scarcely suffice, for we cannot imagine that any incompatibility or failure of fusion of the maternal and paternal plasms or chromosomes could almost simultaneously bring about the disintegration of the tapetal cells. GREGORY (**9**) suggests that "it seems more likely that sterility of the male organs is the expression of some deeper-lying phenomenon affecting the whole plant," and a similar view is held by TISCHLER (**31**). Thus it appears that the cause must be sought in some more wide-spread phenomenon in the hybrid, causing general lack of nutrition of the parts which degenerate; hence the tapetum and pollen mother cells degenerate simultaneously and for the same reason. Recent papers, which need not be referred to in this connection, have also shown that hybridism may cause abnormalities or failure of development in the female gametophyte, though this is apparently less common. These facts, together with the usually normal and in some cases increased luxuriance of the sporophyte, point to the gametophyte as that part of the life history which suffers chiefly from "lack of nutrition." The sporophyte generation can evidently be carried through successfully if fertilization is once accomplished; but something which first displays itself as lack of nutrition (as shown by the failure of the tapetum to function, as well as perhaps by the pale staining of the chromatin of the pollen mother cells in some cases) prevents the normal development of the gametophyte.

That this is not the whole case, however, is shown by the fact that the reduction divisions are abnormal, even when the tapetum appears perfectly developed and functional. Hence something other than "lack of nutrition" must be the cause of these abnormalities in development. The ingrowth of the median wall layers to fill the loculus after the disappearance of the pollen, points rather in the opposite direction. But perhaps a harmony of these two points of view may be reached. If the view, recently emphasized by COOK and SWINGLE (**6***a*), ALLEN (**1**), CARDIFF (**6**), and others among botanists, may be accepted, namely, that the maternal and paternal chromatin, which contribute to the fusion nucleus at fertilization, maintain their separate identity throughout the sporophyte generation, so that the act of fertilization is really only completed with the fusion of the spirem threads in synapsis, as a preliminary to sporogenesis; then, as these authors point out, the synapsis stage becomes the critical point in the life cycle of the individual. It is conceivable that the maternal and paternal chromatin after entering the same nucleus in fertilization could continue in this condition throughout the sporophyte history without interfering with a development, but that the intimate union which occurs in synapsis, where an interchange of material between the parental idioplasms probably takes place, might lead to the development of "incompatibilities" between the plasms, such as are exhibited in irregularities and disturbances in the reduction divisions, and finally more or less complete failure of later development. It may be remembered in this connection that in *O. lata* no irregularities in the prophases were observed until after the contracted condition of the spirem.

This discussion is intended to serve merely as a partial analysis of the problem, of which it does not offer a solution. A larger body of facts is very desirable, correlating cytological studies of hybrids with observations of their variations and their relations to the parental forms in all their characters. It may then be possible to determine whether observable differences in the mechanism of inheritance as exhibited by the chromosomes occur in Mendelian, intermediate, variable, and other types of hybrids.

THE CHROMOSOMES AND MUTATION

It is believed that one of the most important discoveries connected with the investigation thus far has been the observation of certain chromatic bodies with a peculiar origin and history, which have been designated heterochromosomes. Since they have been found in the dominant *Lamarckiana* × *lata* hybrid, I believe it is probable that they will also appear in *O. Lamarckiana* itself. This point is to be investigated as soon as possible. If this surmise is found to be true, then the pollen mother cells in which these bodies occur must differ in chromatin content, and hence probably in hereditary value, from those in which they are absent, provided that pollen grains from both these classes of mother cells reach maturity. It is highly probable that the latter supposition is true in the *O. Lamarckiana* hybrid, for an abundant amount of pollen reaches maturity, so that in crossing experiments fertilization is as easily accomplished as with pure *O. Lamarckiana* pollen; which increases the probability that no extensive deterioration of pollen grains occurs. POHL (22) states, however, that only about 70 per cent. of the pollen grains of *O. Lamarckiana* are perfect. Whether the deterioration is selective is of course not known.

The fact that the heterochromosomes originate from the spirem by the cutting off of a portion of its length after synapsis, and later degenerate, suggests that in this manner a portion of the chromatin is dispensed with in certain pollen mother cells, while in others where heterochromosomes do not occur it is all retained. Those who hold strictly to the individuality of the chromosomes, and that they represent in some way certain specific characters or groups of characters, would probably maintain that by this means the specific characters represented by the discarded chromatin are lost. LILLIE (15) expresses the view (which he strongly substantiates) that the chromosomes can represent only general characters belonging to the organism as a whole; such as color, stature, pubescence, etc. These would appear to correspond with the unit characters of DEVRIES, at least in many essentials, for instances of the latter as cited by DEVRIES in the Oenotheras are pigment (in *O. rubrinervis*), stature (*O. nanella* is a dwarf), and pubescence. This loss of chromatin in the germ cells might then appear to be a method of dispensing with certain unit characters of the organism. A great amount of additional work will be required, however, in the study of somatic characters in connection with chromosome morphology, before it will be possible to correlate these two categories of structure, if such a correlation exists.

ROSENBERG (25) has recently published a further paper on the Drosera hybrid, in which he finds that occasionally two pollen grains of a tetrad have the characters of one parent and two those of the other parent, the supposition being that this is preceded by segregation of the maternal and paternal chromosomes in the first reduction division. This may be considered a proof that the segregation of parental characters when it occurs takes place during the reduction divisions. In the light of such facts it seems highly probable that mutations in the Oenotheras will be found to originate during the reduction mitoses, and perhaps from irregularities in the distribution of chromosomes. The results of the paper mentioned above would also appear to show that a relation exists between chromosome morphology and the morphology of the pollen grains.

The origin and early stages of some of the bodies classed as "idiochromosomes" by WILSON (36) are similar to those I have found in Oenothera. In *Lygaeus turcicus*, one of the Hemiptera, a large and a small idiochromosome are found in the prophase, the large one first appearing about the time of synapsis. Slightly later it is seen as a more deeply staining longitudinally split thread among the chromosomes, which are also thread-like bodies at this time. During this period a smaller, somewhat elongated idiochromosome is also present. The larger idiochromosome condenses to a globular shape and is for a time attached to the plasmosome, but retains a central cavity, which represents the original longitudinal split. This makes its appearance quite similar to those described in Oenothera. The later history of the idiochromosomes in Lygaeus and other Hemiptera however, is quite different from those of Oenothera, and need not be taken up here. Small chromosomes, which possibly correspond with WILSON'S *m*-chromosomes, are also occasionally found in Oenothera (*figs. 26, 27*).

Miss STEVENS (28) has described chromatic bodies in the insects which correspond with the heterochromosomes of Oenothera in parts of their history. In Stenopelmatus, the "sand-cricket," a chromatic "element *x*" appears in synapsis in the spermatocytes attached to an end of the spirem. It passes undivided into one of the spermatocytes of the second order and there degenerates during the resting stage between the maturation mitoses. Miss STEVENS believes this to be rejected chromatin, analogous to that observed in the ovogenesis of many forms. In the animal Sagitta, which is hermaphrodite, Miss STEVENS (27) found in spermatogenesis bodies which resemble the heterochromosomes of Oenothera in some respects. This "element *x*" appears in the spermatogonial divisions, where it divides before each mitosis. It also divides in the prophase of the first maturation division, and when the chromosomes are on the equatorial plate in metaphase these bodies are found in various positions on the spindle. But they enter the daughter nuclei and divide again before the second spindle forms, and one of the products thus enters each spermatid. No such body is found in ovogenesis.

The only previous suggestion of heterochromosomes in plants is in a recent paper of CARDIFF (**6**), in which he mentions a heterotropic chromosome in the heterotypic mitoses of *Salomonia biflora*. He believes that this chromosome passes to one pole undivided in the first mitosis, but has not studied it further.

The difference found in the number of chromosomes in the mutants of Oenothera very strongly favors considering these forms of "specific" rank. I think it will be evident to any one studying carefully and comparing the different mutants that they are quite as distinct and easily distinguishable as are the species of any ordinary genus. The differences in the number of chromosomes is still further and, I think, conclusive evidence that the forms concerned are distinct "species."

It seems necessary to conclude, therefore, that the phenomena of mutation as described by DEVRIES in the genus Oenothera are either due to *O. Lamarckiana* being some peculiar type of hybrid in which the earlier crosses are appearing again in comparatively rare numbers; or on the other hand that some process of differentiation, the most probable seat of which is the germ plasm, has led to the production of distinct types of germ cells differing in chromosome morphology and in hereditary value. The middle ground, the assumption that these various mutants are merely the widely fluctuating variations of the original *O. Lamarckiana*, is believed to be no longer tenable.

Moreover, there are several lines of evidence, partly negative, which tend in various ways to discredit the possible hybrid origin of these forms. Some of these arguments will be mentioned.

1. It has been clearly shown by MACDOUGAL (**17, 18**) and a number of collaborators that *O. Lamarckiana* was originally native to various parts of North America.

2. If the mutants always existed together with the parent form, it seems probable that some at least would have been recognized as species and preserved in European or American herbaria.

3. *O. biennis* and *O. grandiflora*, American members of the genus, have been shown by MACDOUGAL to be mutating.

4. Among all the known types of hybrids, with their widely varying methods of transmission and of variation, none are known, so far as the writer is aware, which present a series of phenomena of variation resembling or comparable to those found in *O. Lamarckiana* and its mutants.

5. Hybrids from crosses among the mutants of Oenothera follow the ordinary laws of hybridization; *e. g.*, *O. lata* × *O. Lamarckiana* is a Mendelian or dichotomous hybrid; and *O. nanella* × *O. rubrinervis* is a hybrid of intermediate type, which breeds true.

6. If *O. Lamarckiana* has itself been hybridized, where did the forms originate with which, on this assumption, it was hybridized? No scrap of evidence that they ever originated from any source except from *O. Lamarckiana* seed has been found. We must then assume that this is their only method of origin until the contrary is proved to be the case.

Summary[5]

1. Failure of pollen development in *O. lata* is not due to ingrowth of the tapetum to fill the loculus, as described by POHL, but to some other agency at work in the hybrid, the nature of which is not fully explained.

2. Pollen development in *O. lata* may proceed to the formation of tetrads, but degeneration of both mother cells and tapetum frequently begins in the resting stage or during the prophase of the first mitosis.

3. Heterochromosomes arise in *O. lata* in the prophase after synapsis. There may be one or two such bodies, which are formed as large rings by the cutting off of a portion of the spirem thread before the remainder breaks up into chromosomes. These bodies later are found in metaphase of the heterotypic mitosis, usually in the cytoplasm near the spindle. They do not divide, but become smaller and

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[5] As this paper is passing through the press, conditions are being found in the pure *O. Lamarckiana* which will modify the interpretation of the bodies called heterochromosomes. The inference that *O. Lamarckiana* itself has the same number of chromosomes as the dominant *O. Lamarckiana* hybrid is also apparently not borne out by the facts. There seem to be variations in the number of chromosomes, which will require further careful study before an interpretation will be possible.

probably disappear at the end of the first mitosis. These bodies are also found in the *O. Lamarckiana* hybrid, in which they doubtless have the same origin. They probably represent discarded chromosomes, and this is perhaps a means of lessening the number of chromosomes in certain germ cells of the species. Some mother cells do not contain them. In such cells the (sporophyte) count of chromosomes in *O. lata* is fourteen and in the *O. Lamarckiana* hybrid probably twenty.

4. The occurrence of extra nuclei formed by chromosomes left behind in the cytoplasm in the tetrad divisions of the hybrid *O. lata*, as well as in a number of other hybrids recently described, puts under suspicion the purity of any other plant, such as *Hemerocallis fulva*, in which such extra nuclei are known to occur.

5. The inference seems justifiable that the mutations of *O. Lamarckiana* arise during the reduction divisions and that pollen grains which will give rise to mutants differ in their potentialities and probably also in chromatin morphology from the ordinary pollen grains of the plant.

These facts lead to the conclusion that a cytological basis may be found for the phenomena of mutation as observed in the Oenotheras.

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EXPLANATION OF PLATES II–IV

All the figures were drawn with the aid of a Bausch and Lomb camera lucida. A Zeiss 2^{mm} apochromat oil immersion lens was used, with Zeiss compensating oculars. The figures are all reduced one-half.

PLATE II

Oenothera lata (dominant)

FIG. 1. Transverse section of a loculus of an anther; pollen mother cells in "resting" stage. ×325.

FIG. 2. Slightly later stage; pollen mother cells passing from "resting" condition to early prophase; tapetal cells uninucleate. ×690.

FIG. 3. Later stage; pollen mother cells degenerating; tapetal cells binucleate, some of them degenerating. ×690.

FIG. 4. Pollen mother cell degenerating; the nucleus has disappeared and irregular deeply staining bodies appear in the cytoplasm. ×1500.

FIG. 5. A loculus after the pollen mother cells have disappeared; the remnants of the tapetum form a thin layer partly lining the cavity; the ingrowth of the middle layers has begun. ×325.

FIG. 6. A later stage; the middle layers have grown in, almost completely obliterating the loculus. ×325.

FIG. 7. Late stage in pollen development; the middle layers are being pressed together; the tapetal cells are narrow radially; the pollen grains are very irregular in shape. ×155.

FIG. 8. "Resting" nucleus of pollen mother cell; the nucleolus contains several highly refractive vacuoles; at the nodes of the reticulum are chromatic bodies, probably representing the "prochromosomes" of OVERTON. ×2125.

FIG. 9. Slightly later stage; the chromatic bodies are rounded in shape; safranin-gentian; 3.3 μ. ×2125.

FIG. 10. Slightly later stage; the reticulum is beginning to contract away from the nuclear membrane, which at this time is extremely delicate; 3.3 μ. ×2125.

FIG. 11. Spirem stage just previous to synapsis; four nucleoli are present; the nuclear membrane is extremely delicate; the spirem thread is very thin, about half its thickness subsequent to synapsis; Safranin-gentian; 4 μ. ×2125.

FIG. 12. Spirem thread contracted in synapsis; iron-haematoxylin-orange; 5μ. ×2125.

FIG. 13. Mother cell in synapsis; chromatic masses are scattered through the cytoplasm; iron-haematoxylin-orange; 7.5 μ. ×2125.

FIG. 14. Just after synapsis; loops of the spirem pass outwards to the wall, giving a false appearance of doubling; the nucleolus is flattened and appressed to the nuclear wall, which is more definite at this time; iron-haematoxylin-orange; 5 μ. ×2125.

PLATE III

FIGS. 15–24. *O. lata* (dominant).

FIG. 15. Nucleus at the time of synapsis; the nuclear membrane is very thick and irregularly indented; safranin-gentian; 4 μ. ×2125.

FIG. 16. Portion of spirem after synapsis; it is about double its former thickness and very much shorter; the loop (*a*) of the spirem may indicate the method of origin of a heterochromosome; safranin-gentian; 4 μ. ×2125.

FIG. 17. Slightly later stage showing a portion of the spirem after synapsis; the large ring body, which has been detached from the spirem, is a heterochromosome (*h*); the nucleolus is present and the nuclear membrane is becoming delicate previous to breaking down; safranin-gentian; 3.3 μ. ×2125.

FIG. 18. Section of nucleus showing two nucleoli and a heterochromosome (*h*); the adjacent section contains the spirem; safranin-gentian; 4 μ. ×2125.

FIG. 19. Similar section showing two heterochromosomes (*h*) and the nucleolus; safranin-gentian; 4 μ. ×2125.

FIG. 20. Late prophase, showing nucleolus, two heterochromosomes (rings), and seven other chromosomes with indications of pairing; safranin-gentian; 3.3 μ. ×1500.

FIG. 21. Later prophase showing thirteen chromosomes; the nucleolus and one chromosome on adjacent section; no heterochromosomes present; indications of pairing of the chromosomes; the cytoplasm is pushing into the nuclear cavity; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 22. Nucleolus and ten chromosomes; the stain is withdrawn from the shell of the nucleolus, except on one side; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 23. Metaphase of heterotypic mitosis, showing twelve chromosomes (some paired) and a heterochromosome (*h*); 5 μ. ×1500.

FIG. 24. Same stage as last, showing two heterochromosomes (*h*) in the cytoplasm, and ten chromosomes on the spindle; 5 μ. ×1500.

FIGS. 25–34. *O. Lamarckiana* (dominant).

FIG. 25. Section of heterotypic spindle, showing two heterochromosomes (*h*) of equal size on the same end of the spindle; 7.5 μ. ×1500.

FIG. 26. Same stage, showing chromosomes closely grouped in the equatorial plate; two heterochromosomes (*h*) of unequal size are present, and a very small chromosome (*m*) out of position on the spindle; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 27. Showing chromosomes closely grouped in the equatorial region of the spindle, and a small chromosome (*m*) nearer the pole; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 28. Showing two smaller paired chromosomes (*m*) at the side of the spindle; 7.5 μ. ×1500.

FIG. 29. Polar view of equatorial plate of heterotypic mitosis; one heterochromosome (*h*) present; 7.5 μ. ×1500. *Fig.* 32 is the adjacent section of this nucleus.

FIG. 30. Section of heterotypic spindle, showing a heterochromosome (*h*), an unequal pair of chromosomes, and an equal pair at the periphery of the cell. 7.5 μ. ×1500.

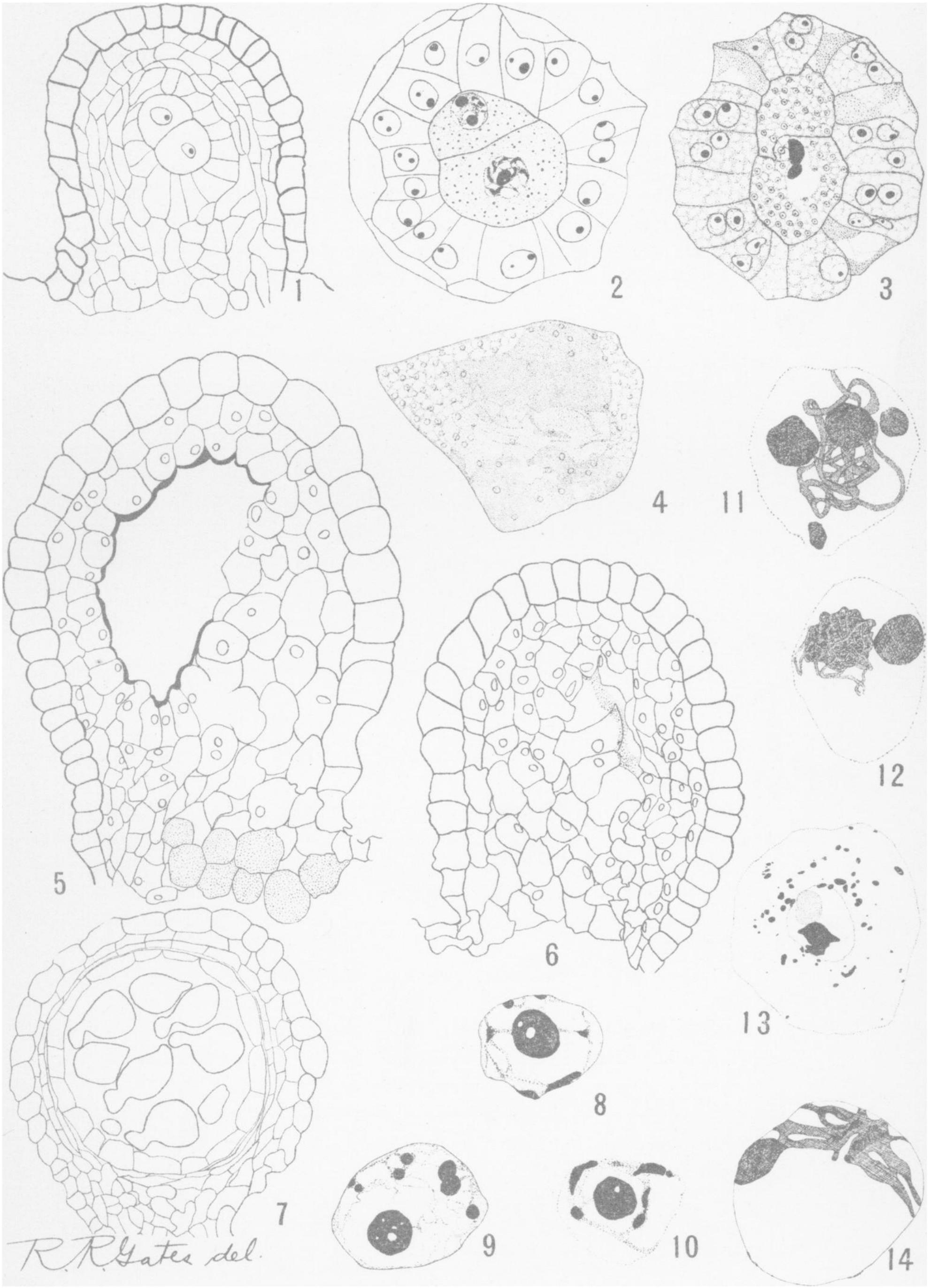
FIG. 31. Portion of the spindle, showing two chromosome dyads and a heterochromosome (*h*); 7.5 μ. ×1500.

FIG. 32. Portion of the equatorial plate, showing two very small bodies which stain less deeply than the chromosomes and are probably remnants of a heterochromosome; iron-haematoxylin-orange; 7.5 μ. ×1500.

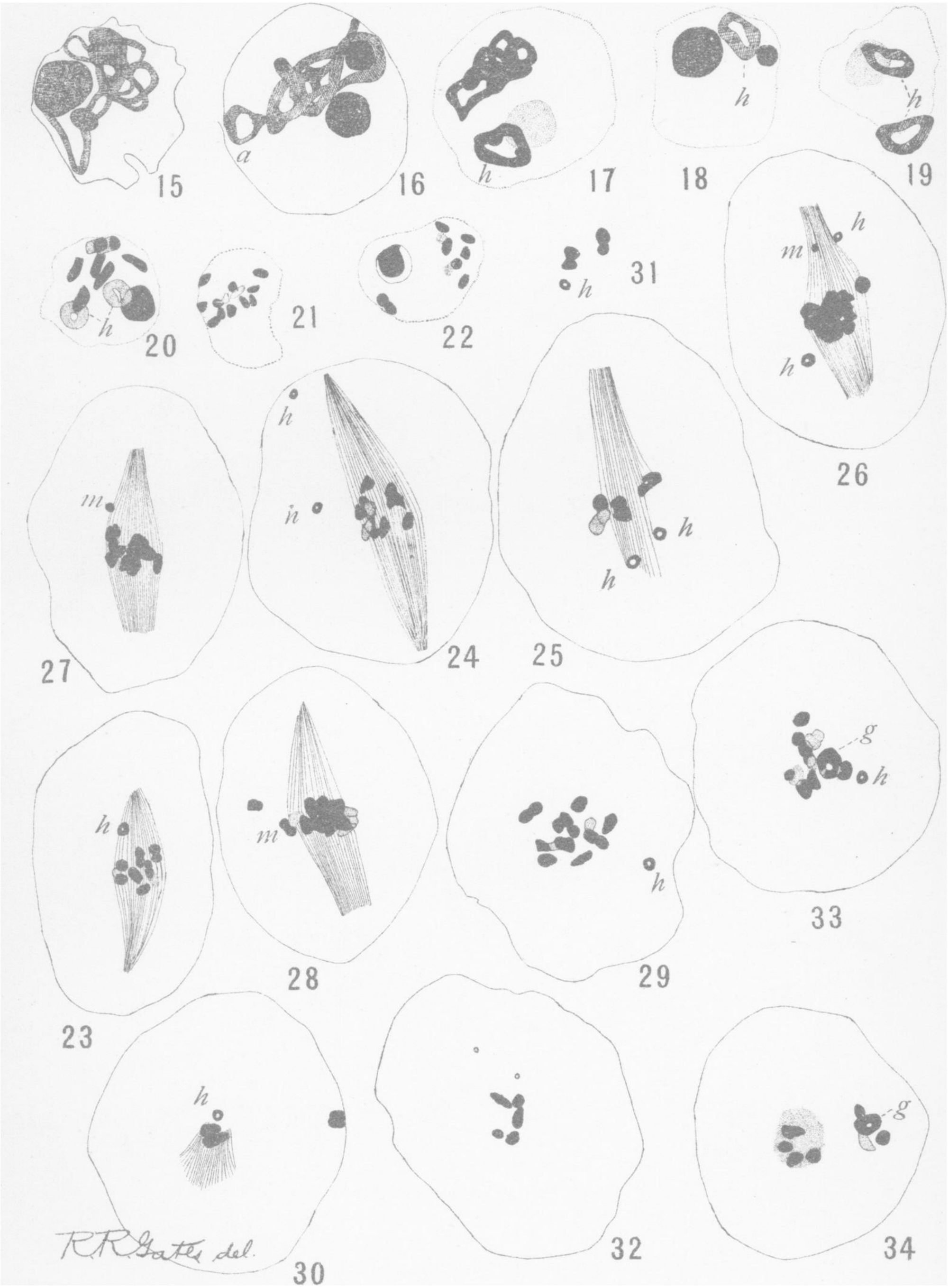
FIG. 33. Section of equatorial plate of heterotypic mitosis, showing a number of chromosomes, a heterochromosome (*h*), and a giant chromosome (*g*), which is very large and ring-shaped; 7.5 μ. ×1500.

FIG. 34. Equatorial view of heterotypic spindle; the chromosomes at the side are displaced in the figure for the sake of clearness; one of them is the giant chromosome (*g*); 7.5 μ. ×1500.

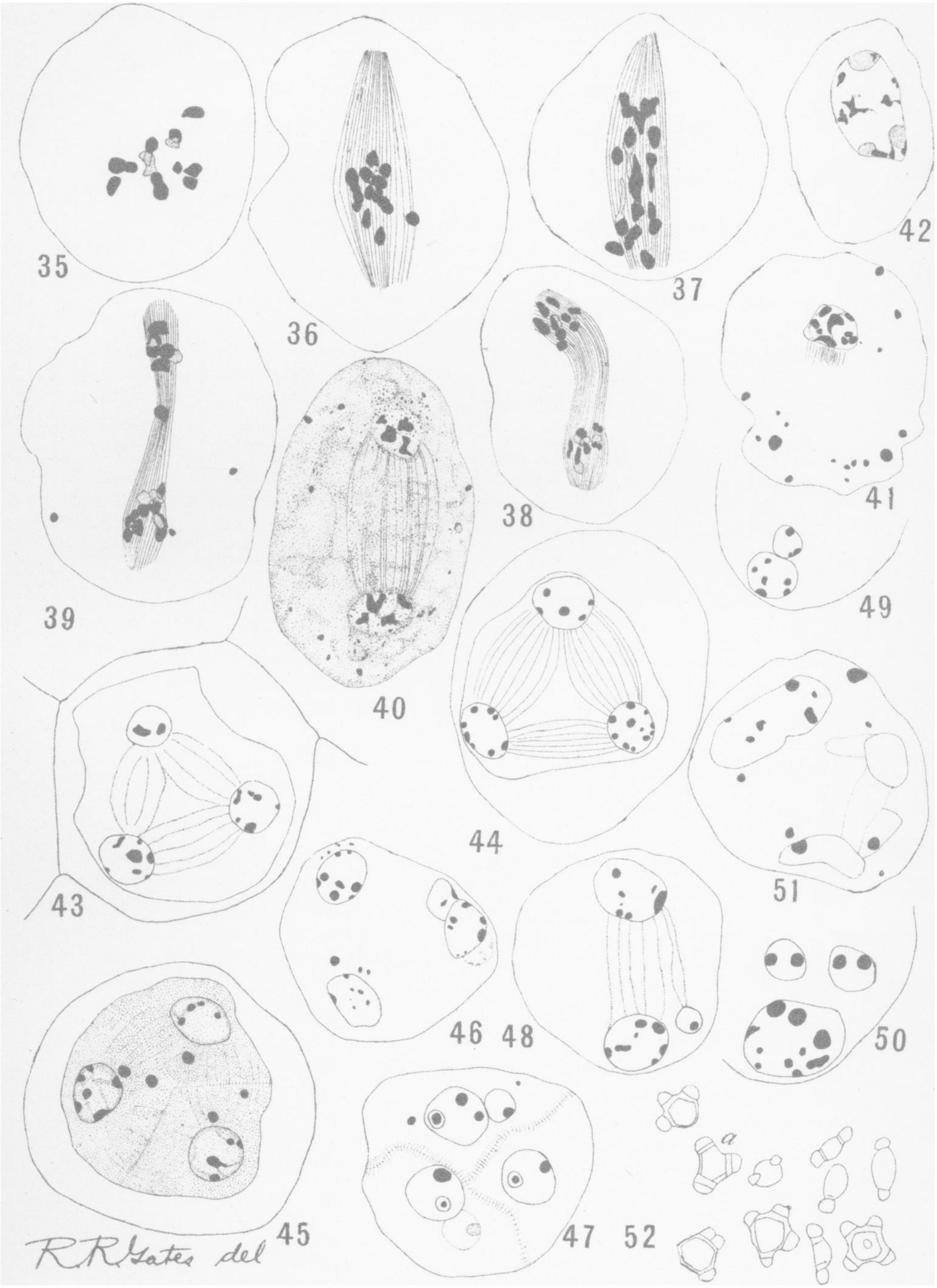
PLATE II



GATES on OENOTHERA



GATES on OENOTHERA



GATES on OENOTHERA

PLATE IV

FIGS. 35–42. *O. Lamarckiana* (dominant).

FIG. 35. Another equatorial view; the unequal appearance of the members of a pair may be due to foreshortening. × 1500.

FIG. 36. Heterotypic spindle showing tendency of chromosomes to arrange themselves in groups of more than two. × 1500.

FIG. 37. Early anaphase, showing viscid consistency of some of the chromosomes; the number is at least twenty; no heterochromosomes present; iron-haematoxylin-orange; 7.5 μ. × 1500.

FIG. 38. Late anaphase, showing premature splitting of the chromosomes for the homotypic division; twenty chromosomes present; 7.5 μ. × 1500.

FIG. 39. Late anaphase; one chromosome lagging behind on the spindle, and several chromatic bodies in the cytoplasm; 7.5 μ. × 1500.

FIG. 40. Telophase in which the reconstruction of the daughter nuclei has begun; 7.5 μ. × 1500.

FIG. 41. One of the daughter nuclei in the early stages of reconstruction; remnants of the spindle still connect it with the sister nucleus; several large chromatic bodies are found in the cytoplasm; 7.5 μ. × 1500.

FIG. 42. Polar view of daughter nucleus in late stage of reconstruction; iron-haematoxylin-orange; 10 μ. × 1500.

FIGS. 43–51. *O. lata* (dominant).

FIG. 43. Pollen tetrad, showing three of the four nuclei; the latter vary in size and in chromatin content; the tetrads are still attached to each other at the ends, but free laterally; 5 μ. × 1225.

FIG. 44. Same as last, but nuclei of nearly equal size; safranin-gentian-orange; 5 μ. × 1275.

FIG. 45. Tetrad showing first appearance of walls; chromatic bodies in the cytoplasm; 5 μ. × 1225.

FIG. 46. Tetrad showing two extra nuclei attached to one of the daughter nuclei; 5 μ. × 1275.

FIG. 47. Tetrad after wall formation, showing extra nuclei, and chromosomes in the cytoplasm not surrounded by a nuclear membrane; 8 μ. × 1225.

FIG. 48. Section of a tetrad, showing a small extra nucleus, formed by one chromosome, connected by spindle fibers with one of the larger nuclei; 5 μ. × 1225.

FIG. 49. Portion of a tetrad showing a small extra nucleus beside one of the tetrad nuclei; 5 μ. × 1225.

FIG. 50. Portion of tetrad showing two small extra nuclei, each containing two chromosomes; 5 μ. × 2125.

FIG. 51. Tetrad showing irregular nuclei, and chromatin in the cytoplasm; 8 μ. × 1500.

FIG. 52. Young pollen grains of *O. Lamarckiana* (dominant); *a* shows a normal-shaped grain; grains showing various abnormalities, such as those illustrated, are of frequent occurrence. × 310.